Sortase A

Week 16

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 Testing conjugation between spider silk and a peptide

Responsible

Oscar Frisell

Protocols used

Sortase A Conjugation
SDS-PAGE

Modifications and comments to protocols

The reaction proceeded for 30 minutes at 37 °C instead of 3 hours at room temperature.

Experimental Data

Samples: 10 ml of 521 μM Z_{Q32C}-SR-H_6 was dissolved in 1x Sortase A binding buffer.
25 ml 10x Sortase A binding buffer
2 tubes with 50 μl of 1mM Sortase A in Sortase A storage buffer.
30 mg/ml Spider silk
Calculations
Total volume = 550μl.
Amount of Z_{Q32C}-SR-H_6 = 230 μl $\Rightarrow$ (c_2*V_2)/(c_1)=(n_2)/(c_1)=V_1=120nmol/521μl = 230 μl.
Amount of Sortase A Binding buffer= 55μl $\Rightarrow$ 1x in final volume, 10x in stock, (1x/10x)*Total volume= 0.1*550= 55μl
Amount of Sortase A = 2.75 μl $\Rightarrow$ V_1=(5μM*550μl)/1mM=2.75μl
Amount of Spider silk with N-terminal= 231μl $\Rightarrow$ M_w=22.997kDa
Concentration = 3 mg/ml= 130 nmol/ml
Volume = (30nmol)/ (139nmol/ml) = 231 μl

Results and Conclusions

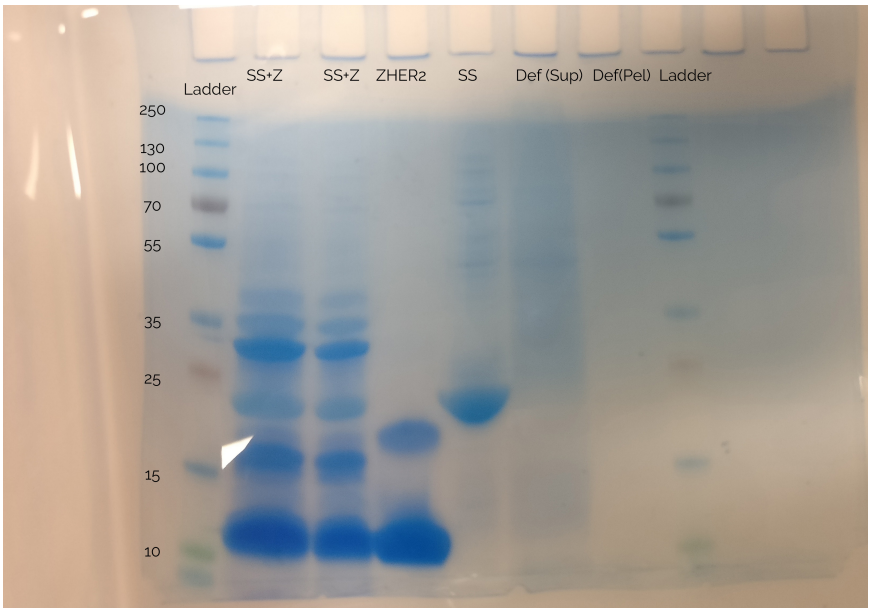

Figure 1: SDS-PAGE with samples. SS+Z is spider silk with conjugated protein Z. Well 7-8 is not of interest.

Discussion and Troubleshooting

The result seen in Figure 1 have shown that the spider silk has been fused with one or multiple protein Z which means the conjugation has worked. The size of spider silk can be seen in well 5 and it also exist in well 2 and 3. In well 2 and 3 it is additionally proteins around the 35 kDa line which is the conjugated proteins. The theory of that Sortase have the capability to conjugate two proteins together is correct.

2 Site directed mutagenesis for removal of XbaI restriction site

Responsible

Aman Mebrahtu

Protocols used

PCR Amplification using Q5 Polymerase

Modifications to protocols

No modifications were made to the protocol. Two sets of primers were used, one set with Back to Back primers and the other designed to overlap. Both primers has one nucleotide switched out. Three reactions for each primer pairs were carried out.

Experimental Set up

Table 1: PCR Set up for SDM on Sortase gene.

Q5 Hot Start High-Fidelity 2X Master Mix	12.5 μl	1X
10 μM Forward Primer	1.25 μ	0.5 μM
10 μ Reverse Primer	1.25 μl	0.5 μM
Template DNA (125 ng/μl)	1 μ	1-25 ng
Nuclease-free water	9.0 μl	

Results and Conclusions

Observing the gel, the conclusion was quickly drawn that the Site directed mutagenesis was unsuccessful for both primer pairs. No strong Bands of interest are observed on the gel, though unspecific bands can be observed for the overlap primer-set. The failed attempt is most likely caused by lacking primer design, which needs to be reviewed and possibly changed substantially, e.g. design primer pairs with an higher Tm to avoid unspecific binding.

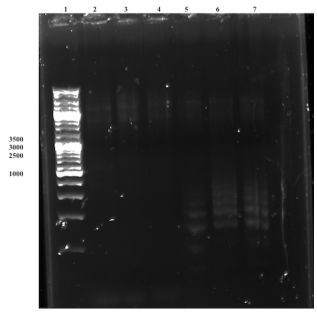

Figure 2: Result after gel electrophoresis in 1 % agarose, at 150 V. (1) DNA Ladder (2) GS-BBP-1 (3) GS-BBP-2 (4) GS-BBP-3 (5) GS-OLP-1 (6) GS-OLP-2 (7) GS-OLP-3.